Topic 3 – Atherosclerosis and inflammation – C

April 24th, Thursday 2014

0380

Truncated thioredoxin (Trx-80) promotes pro-inflammatory macrophages of the M1 phenotype and enhances atherosclerosis

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Vascular cells are particularly susceptible to oxidative stress that is believed to play a key role in the pathogenesis of cardiovascular disorders. Thioredoxin-1 (Trx-1) is an oxidative stress-limiting protein with anti-inflammatory and anti-apoptotic properties. In contrast, its truncated form (Trx-80) exerts pro-inflammatory effects. Here we analyzed whether Trx-80 might exert atherogenic effects by promoting macrophage differentiation into the M1 pro-inflammatory phenotype. Trx-80 at 1µg/ml significantly attenuated the polarization of anti-inflammatory M2 macrophages induced by exposure to either IL-4 at 15ng/ml or IL-4/IL-13 (10ng/ml each) in vitro, as evidenced by the expression of the characteristic markers, CD206 and IL-10. By contrast, in LPS-challenged macrophages, Trx-80 significantly potentiated the differentiation into inflammatory M1 macrophages as indicated by the expression of the M1 cytokines, TNF-α and MCP-1. When Trx-80 was administered to hyperlipoproteinemic ApoE2.Ki mice at 30µg/g body weight (b.w.) challenged either with LPS at 30µg/30g (b.w.) or IL-4 at 500ng/30g (b.w.), it significantly induced the M1 phenotype but inhibited differentiation of M2 macrophages in thymus and liver. When ApoE2.Ki mice were challenged once weekly with LPS for 5 weeks, they showed severe atherosclerotic lesions enriched with macrophages expressing predominantly M1 over M2 markers. Such effect was potentiated when mice received daily, in addition to LPS, the Trx-80. Moreover, the Trx-80 treatment led to a significantly increased aortic lesion area. The ability of Trx-80 to promote differentiation of macrophages into the classical proinflammatory phenotype may explain its atherogenic effects in cardiovascular diseases.

0398

Role of non-muscular myosin light chain kinase (nmMLCK) in the inflammation associated with intermittent hypoxia

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Intermittent hypoxia (IH) alters endothelial function favoring inflammation and could accelerate atherosclerosis-induced cardiovascular diseases. A protein that may play a role in this process is the non-muscular myosin light chain kinase (nmMLCK). The deficiency of this kinase protects mice from death in septic choc models and prevents the atherosclerosis in mice fed with a high fat dietary. The aim of this study was to analyze the implication of nmMLCK in the vascular effects and the inflammation induced by IH. Human aortic endothelial cells (HAoECs) and endothelial cells isolated from wild type or deficient nmMLCK mice were exposed to 6h of IH, which include 30min of hypoxia (O2 5%) followed by 30min of normoxia (O2 21%), in the absence or the presence of ML-7 (5µM), a nmMLCK inhibitor. After the stimulation, we evaluated the production of the superoxide anion, nitric oxide (NO), the pro-inflammatory cytokine IL-6, and also the activation of the NF-κB pathway. IH treatment increased superoxide, NO and IL-6 production in HAoECs. However, while the nmMLCK inhibitor ML-7 had no effect on the IH-induced superoxide anion increase, it decreased both NO and IL-6 production. Furthermore, p65-NF-κB pathway was activated by IH in a ML-7-insensitive manner in HAoECs. By using aortic endothelial cells from nmMLCK deficient mice, we showed that nmMLCK deletion abolished the increase of superoxide anion induced by IH indicating that the presence and not the kinase activity of nmMLCK is crucial to regulate oxidative stress induced by IH. In conclusion, IH induced oxidative and nitrative stresses as well as changes in inflammatory secretome of human endothelial cells. In this experimental model of IH, both nitrative and pro-inflammatory cytokine secretion are sensitive to inhibition of the activity of nmMLCK and oxidative stress require its presence. These results suggest strength the notion that nmMLCK participates to the IH-induced inflammatory process in endothelial cells.

0289

Inflammation is the main predictor of high-on aspirin platelet reactivity in stable vascular patients treated with monotherapy

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Background: High platelet reactivity (HPR) despite antiplatelet treatment is related to ischemic events in patients with coronary artery disease (CAD), but cannot be assessed routinely in all patients. The aim of the study was to identify clinical and biological predictive factors of HPR on aspirin.

Methods: 333 consecutive patients chronically treated with aspirin for CAD or cerebrovascular disease with potential high risk of ischemic events (complex angioplasty, diabetes, recurrence), in whom platelet reactivity was assessed between 2011 and 2013, were retrospectively analysed. HPR was evaluated just before the next aspirin intake, defined as aggregation ≥20% using light transmission aggregometry with arachidonic acid 0.5 mg/mL (LTA) and closure time <165s using collagen-epinephrine PFA. Adherence was systematically assessed before sampling**.**

Results: Median age was 63 years old, 65% were male, with a median BMI of 25.5 kg/m², 28% had diabetes mellitus and 15% moderate to severe chronic renal failure. Median dose of aspirin was 100mg/day (range 75 to 160) and 11% had aspirin twice daily. LTA found HPR in 8.7% patients and PFA in 20.1%. In a multivariate analysis, predictor factors associated with HPR using LTA were fibrinogen (OR: 1.51, p=0.01), CRP (OR: 1.01, p<0.01), vWF level (OR: 1.01, p<0.01) and daily aspirin dose (OR: 0.99, p=0.03). There was a trend for diabetics to be associated with HPR (OR: 1.19, p=NS) and for patients with aspirin twice a day to have lower risk of HPR (OR: 0.93, p=NS). Patients with CRP levels >3.0 mg/mL had a higher rate of HPR (14.6% versus 2.6%, p<0.01) and a negative predictive value of 97.3% using LTA. High CRP levels (>3.0mg/mL) were also associated with a 2.6-fold increased risk of HPR using PFA.

Conclusion: Inflammation is the main predictor of HPR on aspirin in stable CAD and cerebrovascular patients treated with monotherapy. Further clinical investigations assessing platelet reactivity should focus on patients with inflammatory states.

0383

Anti-inflammatory and anti-atherogenic effects of the inflammasome NLRP3 inhibitor, arglabin, in ApoE2Ki mice fed a high fat diet

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Background: This study was designed to evaluate the impact of inflammasome NLRP3 inhibition by the natural product, arglabin, on inflammatory response and atherosclerotic lesion in ApoE2Ki mice fed a high fat Western type diet (HFD).

Methods and results: Arglabin was purified to homogeneity and its chemical identity was confirmed by mass spectrometry. It inhibited IL-1β and IL-18 production in cultured C57Bl/6, Nlrp3+/+ mouse peritoneal macrophages in a concentration-dependent manner with a maximum effect at ~50 nM and EC50 values for both cytokines of ~ 10 nM. In contrast, it has no effect on both cytokines production in C57Bl/6, Nlrp3-/- cultured peritoneal macro-

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phages. Intraperitoneal injection of arglabin (2.5 ng/g of BW, twice daily, 13 weeks) into female ApoE2Ki mice fed a HFD resulted in a decreased IL-1β plasma level vs vehicle-treated mice (4.64 ± 1.43 pg/ml vs 11.61 ± 3.05 pg/ml, $p<0.01$). Surprisingly, arglabin also reduced plasma levels of total cholesterol by ~56% ($p<0.01$) and triglycerides by ~ 55% ($p<0.05$). In addition, arglabin reduced the plasma level of anti-oxLDL antibodies and oriented the pro-inflammatory M1 macrophages into the anti-inflammatory M2 phenotype in spleen and arterial lesions. Finally, marked reductions in mean lesion areas in the sinus (45 ± 19%, $p<0.05$) and whole aorta (60 ± 2%, $p<0.01$) were observed.

Conclusions: Arglabin reduces inflammation, plasma lipids and orients tissue macrophages into an anti-inflammatory phenotype in ApoE2Ki mice fed a HFD. Consequently, a marked reduction of atherosclerotic lesions was observed. Thus, arglabin may represent a new promising drug to treat inflammation and atherosclerosis.